Ants Alter Herbivore Damage and the Arthropod Community on the Common Milkweed (*Asclepias syriaca*)

Ian Grettenberger

Abstract

Ants are important generalist predators that alter both predator and herbivore communities and can therefore indirectly affect the primary producers in an ecosystem. In this study, I evaluated whether excluding ants would change the composition of the arthropod community on 3 different clones of common milkweed (*Asclepias syriaca*) and whether these changes would cascade to the plant level and affect herbivory levels and plant performance. The effect of *A. syriaca* clone on the herbivore community and on the impact of ant exclusion was also examined. Application of a sticky pest barrier around the base of the stem successfully excluded ants from treatment plants. Total spider abundance was greatest for ant-free plants. After subdividing spiders into size and guild groupings, ants reduced the abundances of some groups, while others showed no difference between treatment and control. Coccinellid abundance did not respond to the treatment. The presence of ants increased abundance of the tended aphid *Aphis asclepiadis* and *Lygaeus kalmii* and decreased that of the untended aphid *Myzocallis asclepiadis*. On one clone, ants negatively affected *Danaus plexippus* larvae, while ants did not affect *Liriomyza asclepiadis* across all clones. Herbivore damage, as measured by percent leaf area removed, was affected by ants and accumulated only on the ant-exclusion plants on two clones and on both the treatment and control plants for the third. The increase in damage caused by exclusion of ants did not significantly affect the measures of plant performance assessed, relative growth, leaf loss, and fruit production, across all clones. Ant exclusion did reduce fruit production on one clone. These results provide evidence for the importance of ants to the structure of the milkweed community through direct and indirect interactions, although further long-term research is necessary to accurately assess cascading effects on the plant.

Introduction

The scientific community still does not fully understand the mechanisms that control terrestrial ecosystems and allow the persistence of a "green world" and coexistence of thousands of organisms. This is true in spite of years of research investigating the importance of "top-down" compared to "bottom-up" controls since Hairston et al. published their "green world" hypothesis nearly 50 years ago (Hairston et al. 1960). In a strictly "top-down" controlled world, predators keep herbivore populations in check and are themselves regulated by density-dependent prey availability and competition (Hairston et al. 1960). On the other hand, in a "bottom-up" controlled

world, nutrient availability and other environmental components vary the quantity and quality of food available to successive trophic levels, which in turn control herbivore and predator populations (Sinclair 1975; White 1978).

In reality, nature cannot be divided into ecosystems under top-down or bottom-up controls. Instead, it is likely that in most ecosystems, there exists a balance between the two types of controls and both can coexist. However, the balance is dependent on temporal variation as well as variability in abiotic conditions and different trophic levels (Leibold 1989; Hunter and Price 1992). The effects of top-down and bottom-up controls do not operate independently of each other, but rather interact (Moran and Scheidler 2002). This causes different experimental studies to find differing magnitudes of each type of control in diverse systems (e.g. Roininen et al. 1996; Dyer and Letourneau 1999). Predators have the potential to indirectly affect primary producers through their effects on herbivores and numerous studies have found evidence for this (Schmitz et al. 2000; Halaj and Wise 2000; Terborgh et al. 2001, Trussell et al. 2002). Halaj and Wise (2000) found that generalist predators initiated many trophic cascades, although omnivory by generalists has the potential to dampen trophic cascades into more of a trickle, enhancing the importance of bottom-up effects (Dawes-Gromadzki 2002).

In many terrestrial ecosystems, ants are key generalist predators that can have profound effects on lower trophic levels, including herbivores (Hölldobler and Wilson 1995). The majority of studies examining the direct and indirect effects of ants investigate mutualistic systems in which ants are attracted to plants by the presence of extrafloral nectaries, nest sites, and/or honeydew-producing homopterans (Messina 1981; Whalen and Mackay 1988; Michelangeli 2003; Styrsky and Eubanks 2007). In each situation, the availability and magnitude of benefits for ants can increase their presence on foliage and their foraging activity, but the strength and direction of the effect on the plant host depends on the trade-offs between costs and protection (Bentley 1977; Way and Khoo 1992; Strysky and Eubanks 2007, Grover et al. 2008). By protecting aphids, ants could increase the amount of herbivory a plant experiences (Way 1963). Therefore, ant-attendance of aphids can lead to either positive or negative indirect effects on the plant (Styrsky and Eubanks 2007). Ants can directly affect other invertebrates by preying on them, or they can cause other members of the community to alter their behavior when ants are present, both of which can indirectly affect primary producers (Stamp and Bowers 1996; Rudgers et al. 2003). In the absence of these incentives, ants can also have important indirect interactions in food webs simply because of their abundance and foraging strategies (Way 1963, Atelgrim 2005, Sanders and Platner 2007).

As well as indirectly affecting plants by altering rates of herbivory, predators can induce chemical responses in plants through their effects on herbivores (Stamp and Bowers 1996). Common milkweed (*Asclepias syriaca* L.) is useful for studying chemical responses via indirect effects because of its latex and cardenolide based chemical defenses, which can respond to herbivory (Zehnder and Hunter 2007). These traits and other measures of plant quality vary among different genotypes in plants, altering plant quality and affecting the herbivore and predator community on the milkweed and interactions between the two (Agrawal 2005).

The goal of this study is to determine how ants influence the milkweed arthropod community and how these effects cascade to impact herbivory on the plant and plant performance. I compare the effects of ant exclusion on 3 different genotypes of *A.*

syriaca. The different plant clones provide genetic and environmental variability, which may interact with the top-down effects of the ants. I hypothesize that excluding ants will alter the arthropod community by reducing abundances of other predators and of some herbivores while also indirectly benefiting populations of other herbivores. This effect may vary by clone. In addition, I hypothesize that these changes will indirectly reduce herbivory on the plants, which could cascade to affect plant performance.

Methods and Materials

Study Site and Organisms

The experiment was performed at the University of Michigan's Biological Station in northern Michigan (45°33'31.10"N, 84°40'40.56"W). The study site consisted of an open field with well-drained sandy soil of glacial origin. Caretakers at the station periodically mow and burn the field to prevent succession. Over fifty different clones of *A. syriaca* grow in the field, although the number of ramets per clone varies greatly. *A. syriaca* is a perennial that spreads asexually via rhizomes, producing many genetically identical plants, or ramets, around the center of the clone.

Although milkweed possesses significant chemical defenses, arthropods communities inhabit the patches of milkweed. Some of these are host-specific herbivores of the plants. Several significant herbivores that feed primarily and/or specifically on milkweed are *Rhyssomatus lineaticollis* Say, a weevil (Coleoptera: Curculionidae); *Tetraopes tetrophthalmus* Forster, the red milkweed beetle (Coleoptera: Cerambycidae); *Aphis asclepiadis* Fitch, an ant-tended aphid (Hemiptera: Aphididae); *Myzocallis asclepiadis* Monel, an untended aphid (Hemiptera: Aphididae), *Lygaeus kalmii* Stal, the small milkweed bug (Hemiptera: Lygaeidae); *Danaus plexippus* L., the monarch (Lepidoptera: Nymphalidae) and *Lyriomyza asclepiadis*, leaf mining flies (Diptera: Agromyzidae). A number of predators spend at least part of their time foraging on milkweed. These include reduviids (Hemiptera: Reduviidae), lacewing larvae (Neuroptera: Chrysopidae), syrphid fly larvae (Diptera: Syrphidae), insidious flower bugs (Hemiptera: Anthocoridae), ladybird beetles (Coleoptera: Coccinellidae), spiders (Arananae) and ants (Hymenoptera: Formicidae)

Experimental Setup

In early July 2008, 120 plants were chosen from 3 different clones in the field: clones 15, 26, and 46 (as demarcated by Mark Hunter), henceforth referred to as clones 1, 2, and 3. Forty ramets from each clone were selected based primarily on isolation from other plants and similar flowering phenology. Plants were used that were not or were barely touching other plants, which helped limit ant access to the plants. To limit ant access, I clipped all vegetation within 30 cm of the plants and any vegetation outside of this cutoff that could potentially form bridges. Ramets adjacent to the focal plant were not clipped, but were instead held back from the experimental plant with flags. Vegetation was clipped when necessary over the course of the experiment. Plants in each treatment did not differ in beginning (day 7) height (U=1550.0, n=115, P=.568), beginning number of leaves (U=1461.5, n=115, P=.354), or number of umbels

(U=3250.5, n=115, P=.569), or maximum number of flowering umbels (U=1553.5, n=115, P=.986).

On July 5 (set as day 1), I applied Tanglefoot® (Tanglefoot Co., Grand Rapids, Michigan, USA) to the base of ant-exclusion plants (20 plants per clone) to create a sticky barrier to ant movement onto the plants. I reapplied Tanglefoot as needed throughout the course of the experiment to maintain ant exclusion. Initially, the experimental plan was to investigate the effects of the ant-aphid mutualism using a full factorial design. To attempt this, aphids were added to half of the Tanglefoot plants and to half of the Tanglefoot-free plants on July 6 (day 2). About twenty aphids of various life stages were added by hand with a paintbrush to the uppermost leaves of each plant. The tops of the plants were then bagged to protect them from predation. I removed the bags two days later and added enough aphids to obtain at minimum twenty aphids per plant. Preliminary observations suggested extremely high mortality and an inability of many of the colonies to persist and grow. Due to this difficulty, I did not supplement aphid populations after this point. Log transformed total observations of *A. asclepiadis* were increased by the aphid application treatment (U=2794.0, n=115, P=.003)

Arthropods

On July 13 (day 9), arthropod data were collected for the first time. Many of the herbivores were classified to species, such as *R. lineaticollis, L. kalmii, T. tetrophthalmus*, *A. asclepiadis*, *M. asclepiadis,* and *D. plexippus*. I recorded whether or not the aphids were tended by ants. Leaf miner damage was used as a proxy for the presence of leafminers. This damage was marked with a permanent marker to avoid recounting. Predators were grouped into coarser taxonomic groups. I noted abundances of ants, chrysopid larvae, coccinellid adults and larvae, and spiders. Spiders were divided into different functional groups and were recorded as web-building, jumping, or crab spiders. They were also size-classed into small, medium, large (<2 mm, 2-10 mm, >10 mm). After these initial surveys, data were collected every three days with two exceptions, one in which data were taken again two days later and one in which three days elapsed before data collection. This amounted to a total of 8 days of arthropod censuses on days 9, 11, 14, 17, 21, 24, 27, and 30.

Plant effects

On 11 July (day 7), plant morphology, flowering phenology, and herbivore damage were measured. For morphology and phenology, I recorded height of plant, number of leaves and umbels, and the flowering status of the umbels. To quantify herbivore damage, the leaf damage was divided into 0-5, 5-30, 30-50, 50-70, 70-90, or 90-100 percent categories. Whether or not the top had been browsed by deer and the presence of weevil damage by oviposition in the stem was also recorded. Deer-browsed plants were not included in any analyses, reducing sample size to 115 plants overall with n=56 for the control and n=59 for the ant-exclusion treatment. I marked weevil damage with a permanent marker to avoid recounting. Plant and herbivory data were collected every three days, except for one instance in which data were collected four days after the previous sampling. Data were collected on 8 different days (days 7, 10, 13, 16, 19, 22, 26, and 29).

<u>Statistical analysis</u>
        Arthropod data were collected on 8 different days, but statistical analyses were performed on the sums for all sampling dates for each plant due to low numbers of observations per sampling date. Each arthropod or grouping of arthropods was not tested for an effect of ant-exclusion if the number of plants that had observations was below 10. Aphid data were log transformed to reduce the variance resulting from a few very large values for total observations on one plant. Ant effects were examined using Mann-Whitney U tests across all clones because of non-normally distributed data with ant treatment as the grouping variable. The data file was then split by clone to individually examine each clone for ant effects with Mann-Whitney U tests. Clone effect was tested with a Kruskal-Wallis one-way analysis of variance. All statistic analyses were performed using SPSS, version 15.0, for Windows (2006).

Results

<u>Impacts on Arthropods</u>
        The ant-exclusion treatment successfully reduced the presence of ants on treatment plants ($U=33.5$, n=115, $P< .000$), with only 4 ants observed on treatment plants compared with 1035 ants on control plants. Ants and clone had varied effects on different groupings and individual species of arthropods (Table 1). Ants nearly affected total arthropod abundance significantly across all clones ($U=1311.5$, n=115, $P=.056$). This total did not include aphids because their very high counts would dominate the total. Arthropods were grouped most basically into the guilds of predators and herbivores. Ants did not have a significant effect on either of these groupings, although the negative effect on predators was nearly significant (predators: $U= 1317.0$, n=115, $P=.060$, herbivores: $U=168.0$, n=115, $P=.729$). The herbivore guild included *L. asclepiadis*, *L. kalmii*, *T. tetrophthalmus*, and *D. plexippus*, but this guild, as with total arthropods, did not include aphids.

Table 1. Mean number of observations (±1SE) for arthropods and groupings of arthropods. Means are averages per plant summed over all censuses (days 9, 11, 14, 17, 21, 24, 27, and 30). Ant effect P-values are represented by x=P<.05, xx=P<.01, xxx=P<.001. Clone effect P-values are represented by y=P<.05, yy=P<.01, yyy=P<.001. For individual clones, ant effects are represented by a,b=P<.05, aa,bb=P<.01, aaa,bbb=P<.001.

Arthropod or group	Clone 1 Ant +	Clone 1 Ant -	Clone 2 Ant +	Clone 2 Ant -	Clone 3 Ant +	Clone 3 Ant -
Ants total [xxx]	10.06±2.67 [aaa]	0.05±0.05 [bbb]	13.37±10.40 [aaa]	0.05±0.05 [bbb]	31.37±6.19 [aaa]	0.11±0.07 [bbb]
Coccinellid larvae [yy]	0.50±0.51 [a]	0.20±0.21 [a]	0.11±0.11 [a]	0.00±0.00 [a]	1.58±1.27 [a]	0.74±0.54 [a]
Coccinellid adults	0.28±0.11 [a]	0.60±0.16 [a]	0.36±0.13 [a]	0.45±0.22 [a]	0.37±0.12 [a]	0.79±0.35 [a]
Anthocorids	0.44±0.25 [a]	0.15±0.08 [a]	0.05±0.05 [a]	0.00±0.00 [a]	0.11±0.11 [a]	0.26±0.17 [a]
Web spiders small [xx, yy]	2.00±0.55 [a]	2.95±0.65 [a]	0.74±0.25 [a]	1.65±0.36 [b]	2.63±0.72 [a]	3.58±0.68 [a]
Web spiders medium [y]	1.39±0.38 [a]	1.85±0.42 [a]	1.58±0.46 [a]	0.85±0.28 [a]	0.84±0.32 [a]	0.53±0.26 [a]
Web spiders total [y]	3.39±0.81 [a]	4.95±0.90 [a]	2.32±0.56 [a]	2.50±0.56 [a]	3.47±0.84 [a]	4.11±0.71 [a]
Crab spiders small [xx, yyy]	0.11±0.08 [a]	0.60±0.20 [a]	0.05±0.05 [a]	0.65±0.39 [a]	0.53±0.18 [a]	1.58±0.42 [b]
Crab spiders medium [y]	0.06±0.06 [a]	0.15±0.08 [a]	0.21±0.13 [a]	0.20±0.12 [a]	0.16±0.09 [a]	0.84±0.26 [a]
Crab spiders total [xx, yyy]	0.17±0.09 [a]	0.75±0.26 [a]	0.26±0.15 [a]	0.85±0.44 [a]	0.68±0.19 [aa]	2.42±0.53 [bb]
Small spiders total [xx, yyy]	2.11±0.58 [a]	3.55±0.66 [a]	0.84±0.26 [a]	2.40±0.67 [b]	3.21±0.72 [a]	5.16±0.82 [a]
Medium spiders total	1.44±0.37 [a]	2.00±0.41 [a]	1.79±0.52 [a]	1.05±0.35 [a]	1.11±0.35 [a]	1.37±0.36 [a]
Spiders total [x, yy]	3.56±0.85 [a]	5.75±0.87 [b]	2.63±0.66 [a]	3.45±0.93 [a]	4.32±0.89 [a]	6.53±0.92 [b]
Predators [yyy]	4.89±1.12 [a]	6.85±0.92 [a]	3.21±0.78 [a]	4.05±1.06 [a]	8.32±2.28 [a]	8.53±1.16 [a]
L. kalmii [x]	0.22±0.13 [a]	0.05±0.05 [a]	0.37±0.14 [a]	0.15±0.08 [a]	0.21±0.13 [a]	0.11±0.11 [a]
T. tetrophthalmus [yy]	0.33±0.20 [a]	0.40±0.16 [a]	0.21±0.13 [a]	0.30±0.15 [a]	0.79±0.20 [a]	0.58±0.16 [a]
L. asclepiadis	0.72±0.20 [a]	0.60±0.22 [a]	0.16±0.09 [a]	0.70±0.21 [a]	0.84±0.28 [a]	1.26±0.33 [a]
D. plexippus [y]	0.11±0.11 [a]	0.65±0.19 [b]	0.05±0.05 [a]	0.05±0.05 [a]	0.32±0.22 [a]	0.16±0.16 [a]
M. asclepiadis (log) [x, yyy]	1.74 ±0.12 [a]	1.48±0.13 [a]	0.74±0.11 [a]	0.34±0.10 [b]	0.42±0.08 [a]	0.25±0.10 [a]
A. asclepiadis (log) [x]	0.38±0.18 [a]	0.80±0.21 [a]	0.66±0.22 [a]	1.06±0.21 [a]	0.84±0.25 [a]	0.84±0.23 [a]
Herbivores (-aphids)	1.44±0.35 [a]	1.75±0.28 [a]	0.79±0.20 [a]	1.25±0.33 [a]	2.16±0.50 [a]	2.37±0.49 [a]
Arthropods total (-aphids) [yyy]	6.33±1.27 [a]	8.60±0.95 [a]	4.00±0.87 [a]	5.30±1.25 [a]	10.47±2.42 [a]	10.89±1.25 [a]

Predators included reduviids, chrysopid and syrphid larvae, anthocorids, coccinellid adults and larvae, and spiders. However, only coccinellid adults and larvae and spiders, including all sub-groupings by size and guild other than jumping and large spiders, were sufficiently abundant for analyses. Ants did not significantly affect coccinellid larvae or adults across all clones (larvae: U=1634.0, n=115, P=.829; adults U=1434.5, n=115, P=.143), although coccinellid larvae abundance varied by clone (Fig. 1a). All groupings of spiders analyzed, except for medium spiders, varied by clone. Small web, small crab, total crab, total small, and total spiders were significantly reduced by ants (small web: U=1196, n=115, P=.009; small crab: U=108.5, n=115, P=.034; total crab: U=85.5, n=115, P=.005; total small: U=1090.5, n=115, P=.001; total spiders: U=1231.5, n=115, P=.018) and the reduction of medium crab spider abundance was nearly significant (U=1427.0, n=115, P=.066) (Fig 1b,c). Ant presence did not significantly reduce medium web spiders, total web spiders, and total medium spiders (medium web: U=1532.0, n=115, P=.470; total web: U=1388.5, n=115, P=.136; total medium: U=1624.0, n=115, P=.870) (Fig 1d).
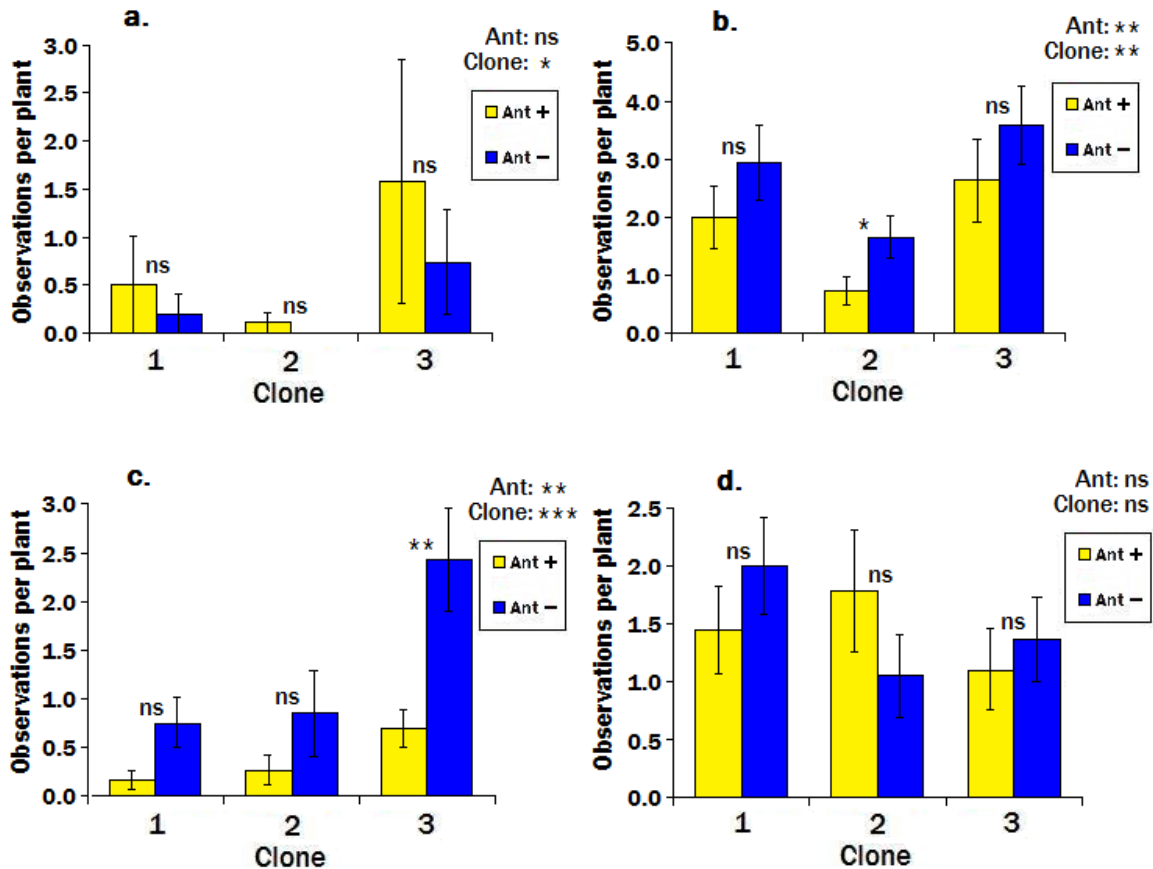
Fig. 1. Effects of ant exclusion on predators. Mean number of observations (±1SE) for **a)** coccinellid larvae, **b)** small web spiders, **c)** total crab spiders and **d)** total medium spiders. Means are averages per plant summed over all censuses (days 9, 11, 14, 17, 21, 24, 27, and 30). Overall ant and clone effects across all clones are indicated above the legend. *P*-values are represented by ns= *P*>.05, *=*P*<.05, **=*P*<.01, ***=*P*<.001. *P*-values for individual ant effects on each clone are represented above their respective bars.

Ants and clone variably affected different herbivores. Several herbivores, *L. kalmii* and *M. asclepiadis*, were more abundant on ant-present plants and there was an overall ant effect on each ($U=1436.5$, n=115, $P=.239$ and $U=1262.5$, n=115, $P=.028$) (Fig 2 a,b). Ants reduced densities of *A. asclepiadis* when combining clones ($U=1309.0$, n=115, $P=.046$), while they only reduced densities of *D. plexippus* when comparing treatments on individual clones ($U=1492.0$, n=115, $P=.136$) (Fig 2 c,d). There was no ant effect, but there was a clone effect on *T. tetrophthalmus* ($U=1623.0$, n=115, $P=.841$; $\chi^2_2=9.552$, $P=.008$) and neither a clone or ant effect on *L. asclepiadis* ($U=1465.5$, n=115, $P=.242$; $\chi^2_2=5.272$, $P=.072$). Ants tending *A. asclepiadis* were observed at some point during the experiment on 10 of the 66 plants that hosted *A. asclepiadis*. *T. tetrophthalmus* was the only herbivore significantly affected by plant height group and were most common on tall plants (3 height groups; small: 36-53cm, medium: 54-64, and large: 65-103) ($\chi^2_2=9.367$, $P=.009$). Plants on which tending occurred had more *A. asclepiadis* and total ants than untended plants (*A. asclepiadis*: $U=90.0$, n=115, $P<.001$; ants $U=97.5$, n=115, $P<.001$).
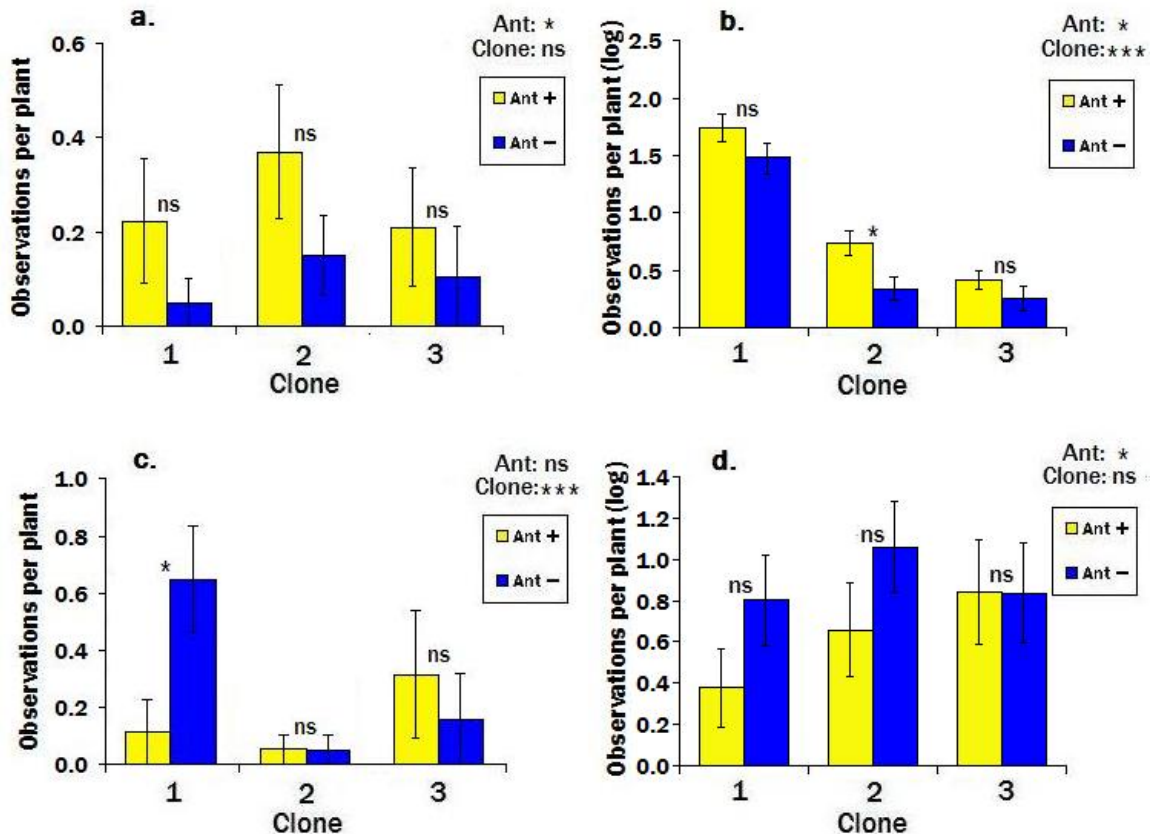
Fig. 2. Effects of ant exclusion on herbivores. Mean number of observations (±1SE) for
a) *L. kalmii*, **b)** *M. asclepiadis*, **c)** *D plexippus*, and **d)** *A. asclepiadis*. Means are
averages per plant summed over all censuses (days 9, 11, 14, 17, 21, 24, 27, and 30).
Overall ant and clone effects across all clones are indicated above the legend. *P*-values
are represented by ns= *P*>.05, *=*P*<.05, **=*P*<.01, ***=*P*<.001. *P*-values for individual
ant effects on each clone are represented above their respective bars.

Impacts on Milkweed

    The change over time in herbivore damage differed between ant treatments for 2
of the 3 clones (see Fig.). Damage was adjusted to starting levels by subtracting starting
levels of herbivore damage from every subsequent measurement. Any positive value for
damage after this adjustment represented new damage, although herbivore damage
measurements could decrease as leaves, both damaged and undamaged fell off. The
exclusion of ants caused damage to significantly increase over the course of the
experiment for clones 1 and 2. On clone 1, damage accumulated on ant-free plants at a
rate of .076% leaf area removed per day (r^2=.897, df=7, P< .001). Damage accumulated
at a rate of .041% leaf area removed per day on ant-excluded plants on clone 2 (r^2=.838,
df=7, P=.001). Herbivore damage on control plants in clone 1 and 2 did not significantly
change over the course of the experiment (1: r^2=.127, df=7, P=.387; 2: r^2=.103, df=7,
P=.439). In contrast, both ant-excluded and control plants accumulated damage over the
course of the experiment in clone 3, with ant-excluded plants accumulating damage at a
rate of .072% leaf area removed per day (r^2=.700, df=7, P=.010) and plants with ants
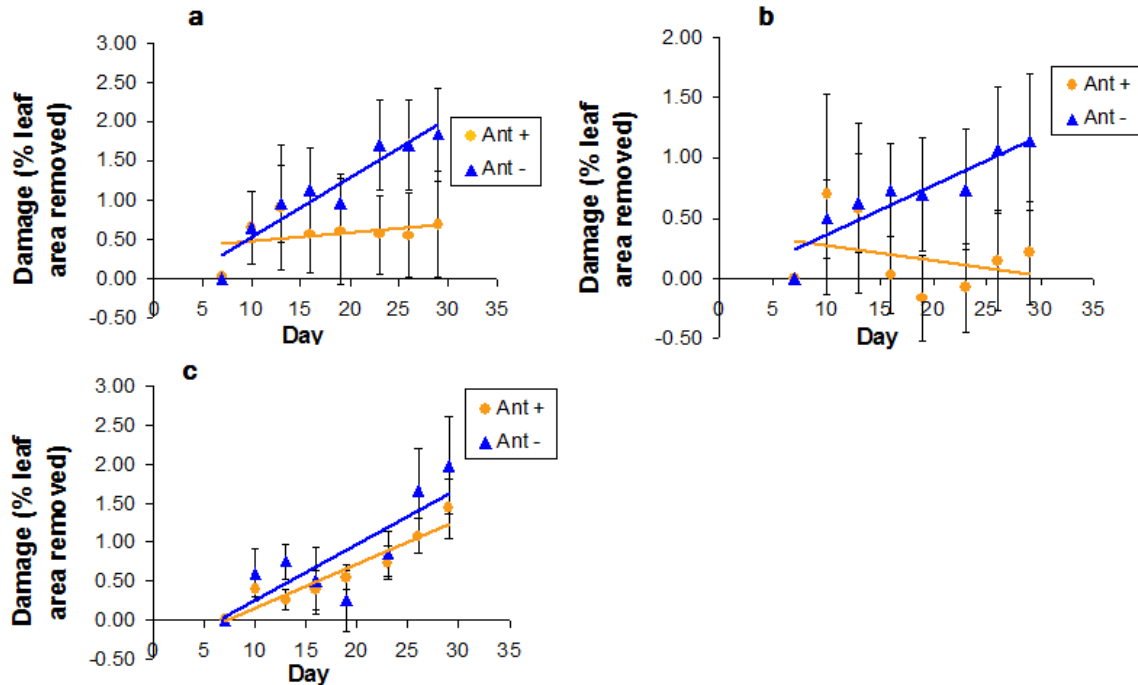accumulating damage at a rate of .057% (r^2=.900, df=7, P<.001).

Fig 3. Change in herbivory over time for **a)** clone 1, **b)** clone 2, and **c)** clone 3. Damage is adjusted 0% on day 7. Subsequent measurements were taken on days 10, 13, 16, 19, 22, 26, and 29.

Ant exclusion minimally affected plant performance. When compared across all clones, ants did not affect relative growth rates (U=1559.5, n=115, P=.605) (Fig 4a). An ant effect on relative growth did not show up on individual clones (1: U=154.5, n=38, P=.460; 2: U=181.5, n=39, P=.813; 3: U=169.0, n=38, P=.751). In addition clone did not affect relative growth rate (χ^2_2=3.933, P=.140).

Ants also did not affect net leaf loss over the course of the experiment (U=1610.5, n=115, P=.816) with clones combined or on individual clones (1: U=159.5, n=38, P=.553, 2: U=161.5, n=39, P=.428, 3: U=150.0, n=38, P=.385) (Fig 4b.). Net leaf loss was calculated by subtracting end leaf counts from the first leaf count data available for each ramet (day 7). This was then adjusted for the total number of leaves per plant by dividing by the average number of leaves, resulting in the units of net leaves lost per leaf. Clone affected net leaf loss (χ^2_2=12.634, P=.002).
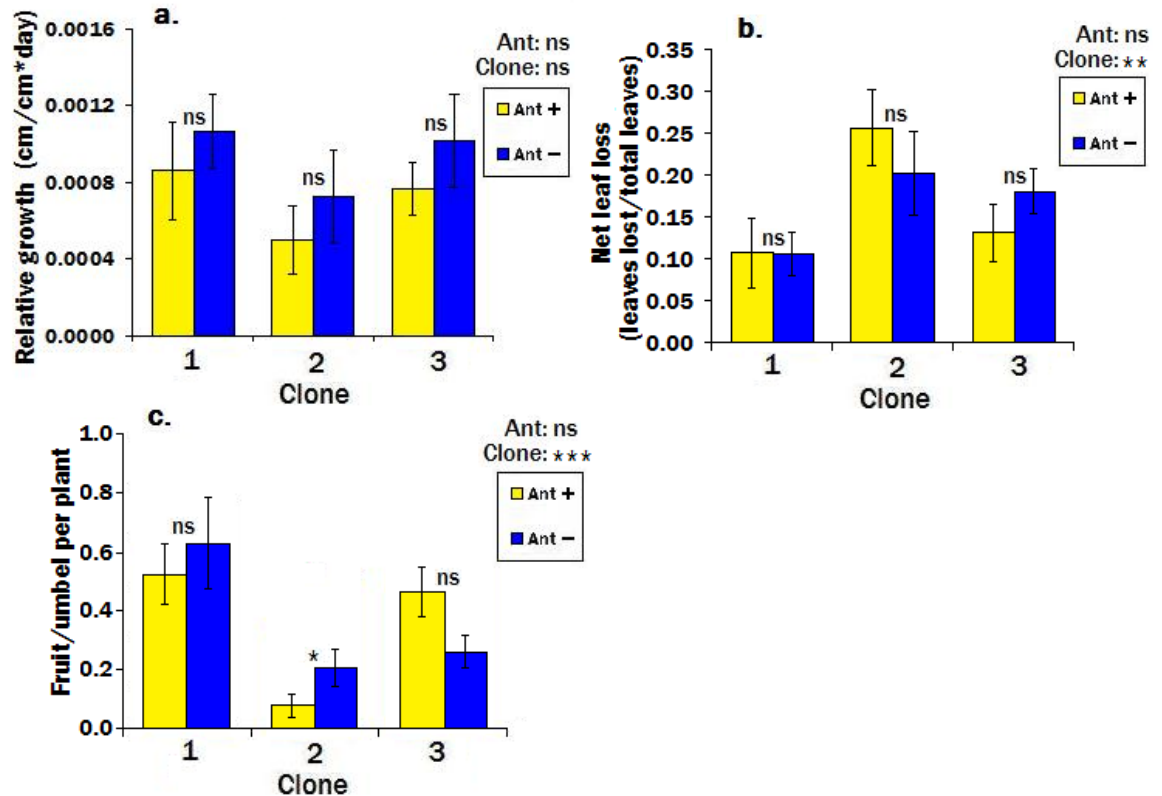
Fig. 4. Effects of ant exclusion on plant performance, as measured by **a)** relative growth rate, **b)** net leaf loss, and **c)** fruit production. Bars are means (±1SE) for each measurement of plant performance. Means are averages per plant for days 7, 10, 13, 16, 19, 22, 26, and 29. Overall ant effect across all clones and clone effects are indicated above the legend. *P*-values are represented by ns= *P*>.05, *=*P*<.05, **=*P*<.01, ***=*P*<.001. *P*-values for individual ant effects on each clone are represented above their respective bars.

Fruit production per plant was corrected for the number of umbels per plant by dividing fruits per plant by umbel counts, yielding fruit/umbel as the units for fruit production. Ants did not significantly affect fruit production across all clones (U=1601.0, n=115, P=.767) (Fig. 4c). Ant exclusion did decrease fruit production on clone 3 (U=102.5, n=38, P=.022), but not clone 1 or 2 (1: U=178.0, n=38, P=.965; *2: U=147.5, n=39, P=.235). Clone affected fruit production (χ^2_2=22.975, P< .001).

Discussion

Overall, the ant-exclusion treatments substantially affected the arthropod community on milkweed, supporting the evidence that ants can be important predators that structure arthropod communities and also supporting the hypothesis that ants would alter the arthropod community (James et al. 1999, Fernandes et al. 2005, Mooney and Agrawal 2008). Not all arthropods responded similarly, which is consistent with other studies and expectations based on different strengths of direct and indirect interactions (Altfield and Stiling 2006). Clone affected both overall abundance and the result of ant exclusion on arthropods. This could result from morphological differences among clones

such as number of leaves, height, flowering phenology, or chemistry (e.g. Osier et al. 2000; Mooney and Agrawal 2008) or it could result from extra-plant biotic and abiotic environmental factors.

The most profound result of ant exclusion on predators was on spiders. Intraguild predation is widespread and commonly occurs between spiders and ants in a diverse set of ecosystems (Polis et al. 1989; Halaj et al. 1997; Sanders and Platner 2007), although this interaction is not universal (e.g. Karhu 1998). Analyzed across clones, ants reduced spider densities in the majority of guild and size groupings, although differences did arise between some groupings. For instance, ant exclusion did not significantly affect medium spiders as a whole, while ant presence decreased small spider abundance, which is a tendency found in another study (Halaj et al. 1997). Spider body size and the susceptibility of different sizes to predation or disruptive interactions may largely determine the interaction between spiders and ants. Grouping spiders into guilds revealed other differential responses of predators to ants. Ants reduced total abundance, including all size classes, for crab spiders while they did not for web-building spiders. Other studies have found both negative and neutral effects of ant exclusion on web-building spiders (Halaj et al. 1997; Sanders and Platner 2007). Web building may reduce the impact of predation by ants on these spiders when compared to other types of spiders, such as crab spiders.

Habitat choice by spiders may also explain differences between guilds. Crab spiders are sit-and-wait predators that inhabit inflorescences and feed on arthropods attracted to the flowers (Morse 1981, De Souza and Martins 2004). The habitat preferences of crab spiders expose them to more encounters with ants compared to other guilds of spiders that do not rely almost exclusively on inflorescences for habitat. This is because floral nectaries on plants, including milkweed, frequently attract large numbers of ants (Fernandes et al. 2005; personal observation). Floral foraging by ants could also explain the magnitude with which ants reduced total spider abundance. Inflorescences attract potential prey and provide refuges from predators, making them attractive to many different types of spiders, although this could enhance conflicts with ants (De Souza and Módena 2004). Although one study did not find a significant effect of inflorescence presence on web-building spiders (De Souza and Martins 2004), another study found that ants expelled the majority of spiders from inflorescences (Faria and Lima 2008). Many web spiders occupied inflorescences during the course of my experiment, suggesting that foliar foraging by ants could negatively influence all spiders and enhance the negative ant effect.

Ants negatively affected herbivores less than predators and positively impacted some herbivores, suggesting the presence of indirect interactions of ants on herbivores mediated by reductions of predators. Both *L. kalmii* and *M. asclepiadis* abundances were greatest in the presence of ants. This may be an indirect effect of ants reducing predator populations. For *M. asclepiadis,* small web-building spiders are likely one of the most important predators counted based on predator and prey size and habitat usage. Ant presence significantly reduced *M. asclepiadis* population individually by clone only on clone 2, which was mirrored by a very significant increase in *M. ascelepiadis* abundance on clone 2 in the presence of ants. Ants did not significantly affect any other arthropods individually on clone 2, except for total small spiders, making this the most likely mechanism that can be suggested from the data collected. The increase in abundance of

L. kalmii in the presence of ants may be evidence of an indirect effect, but care must be taken in interpreting these data because the Tanglefoot treatment could have impeded the movement of *L. kalmii* individuals, despite the fact they fly and did occur on Tanglefoot-treated plants.

The increase in abundance of the untended aphid *M. asclepiadis* and decrease in abundance of the tended aphid *A. asclepiadis* in the presence of ants is counter to previous studies on *A. syriaca* (Mooney and Agrawal 2008). While the effect on *M. asclepiadis* is most likely an indirect effect through changes in predator densities, the effects on *A. asclepiadis* probably result from the costs of the mutualism to the aphid. Ant-aphid relationships described as mutalistic are not always beneficial for the aphid (Billick et al. 2007). Ant tending of *A. asclepiadis* is an important interaction in the milkweed system, large populations of aphids were more likely to be tended than small populations, and counts of ants were greater when they were tending *A. asclepiadis* than when they were not.

Ant effects on the arthropod community on milkweed did translate into changes in the amount of herbivory experienced by the plants, an effect also seen in other studies (Mahdi and Whittaker 1993; Gaume et al. 1997; Karhu 1998; Heil et al. 2001; Sipura 2002). However, ant presence does not always reduce herbivory (Whalen and Mackay 1988; Mody and Linsenmair 2004). Ant exclusion did not consistently increase herbivory across all clones, implicating variable effects by ants among clones on herbivores as an important determinant of the strength of indirect effects of a predator on a plant.

Varying ant and clone effects on abundances of a specific herbivore help explain the accumulation of damage on both ant-present and ant-absent plants in clone 3. The overall ant effect on *T. tetrophthalmus*, one of the most important herbivores causing quantifiable foliar damage, was very insignificant, probably because *T. tetrophthalmus* has very few natural enemies (McCauley and Lawson 1986). There was a significant clone effect and they were much more common on clone 3. Higher abundances of *T. tetrophthalmus* therefore had the potential to drive overall trends in herbivore damage for that clone. The height effect on *T. tetrophthalmus* may have mediated the clone effect on *T. tetrophthalmus* because clone 3 ramets were taller than those of the other clones. Other explanations of habitat effects could explain the attraction of *T. tetrophthalmus* to clone 3, such as differences in the amount of grass around the milkweed plants that the beetles use for oviposition, although this effect pathway cannot be assessed here (Agrawal 2004).

A decrease in fruit production on one clone and a possible trend towards enhanced relative growth in the absence of ants were the only effects on plant performance witnessed. The lack of many significant cascading effects of ant exclusion on measures of plant performance could result from a number of factors. Herbivore damage has been described as a short-term measure of herbivore effects and may merely reflect an increase in herbivore density that does not translate to changes in growth and reproduction (Schmitz et al. 2000; Heil et al. 2001). Even if ant absence increases herbivory, the plant may not respond and no decline in performance is detected (e.g. Stiles and Jones 2001).

The relative brevity of the study in comparison with the growing season of milkweed reduced the ability to detect differences in plant morphology caused by

exclusion of ants. Ant absence considerably affected both the herbivore and predator communities on milkweed. Indirect interactions between ants and other arthropods most likely mediated some of these effects. The altered arthropod community translated into enhanced herbivory on several of the clones, although relatively few repercussions for plant performance accompanied these changes. This suggests that by acting as predators and foraging on foliage, ants are a key element of the milkweed system that determine the structure of the arthropod community. Clone affected the arthropod community and the outcome of the ant-exclusion treatment, emphasizing the importance of considering environmental and genetic heterogeneity when investigating relationships between organisms. Further studies that are more extensive and that incorporate a greater number of precise measurements of plant performance are necessary to fully capture and understand the significant contribution ants make to ecosystems.

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